THE ROLE OF MYCORRHIZAE IN AFFORESTATION[1]

By A. B. HATCH

This paper has large implications. It reports an experiment in which pine seedlings grown in prairie soil were found incapable of normal growth except when artificially provided with mycorrhizal fungi. It has been suspected for many years that mycorrhizal fungi are essential for the normal growth of forest trees. Unless mycorrhizal fungi are introduced in prairie soils through one means or another, the growth of trees planted in them may be unsatisfactory or completely lacking. The extensive program of afforestation in the prairie region as contemplated in connection with the shelterbelt project makes additional research on this problem imperative.

THE suitability of the American prairies for growing "adaptable" trees depends, according to current opinion, on three primary factors: the annual precipitation, the character of the soil, and the adequacy of the cultural and protective care given the plantations during early life.

But 16 nursery and plantation failures have occurred in widely separated regions of the world where these factors were carefully considered and eliminated as contributing agents (10, 1, 14). Moreover in these cases cultivation, fertilization, and watering of seedbeds were not effective in bringing about normal seedling growth. The cause of failure in all cases was eventually traced to the lack of a biological factor in the soils; a factor whose presence is essential to the normal growth of trees in nature.

Descriptions of Nursery Failures

The first failure occurred a decade ago in Western Australia (10). It very nearly caused the abandonment of one of the most extensive afforestation projects on record. At the outset 14 nurseries were started in widely separated areas. The seeds germinated and produced seedlings which were quite healthy during the first few months. Thereafter they became yellowish or purplish, their growth gradually diminished, and finally many died. In the same soils agricultural plants thrived. In a few spots pine seedlings also grew normally, and these were found to possess mycorrhizae, while all other pines lacked them. A few seedbeds were then inoculated with soil containing mycorrhizal fungi. In these the plants soon recovered and showed no further difficulties. Eventually all seedbeds in the 14 nurseries were inoculated, and the project was saved from abandonment. (Kessell, S. L. The dependence of pine on a biological soil factor. Empire For. Jour. 6: 70-74, 1927.)

In the Rhodesian nursery failure (1) the pines grew to a size sufficient for field planting, but they always remained yellow and weak and they never exceeded 4 inches in height. After fertilization and other treatments had failed to bring about improvement, seedbed inoculations were resorted to with spectacular results similar to those first reported from Australia. Mycorrhizal pines from the treated seedbeds were transplanted to the field side by side with the non-mycorrhizal ones of two years earlier. In nine months the mycorrhizal plants had grown to a height of 13 inches while their non-mycorrhizal $2\frac{1}{2}$-year-old neighbors were still less than 4 inches in height. Small quantities of soil containing mycorrhizal fungi

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were then worked into the seedbeds of pine which had been left over in the nursery from the earlier planting: within a month these seedlings turned a dark green and commenced vigorous growth.

In the Philippines the situation was still more striking (14). Attempts were first made in 1909 to extend the natural highland range of *Pinus insularis*—the most valuable softwood in the islands—to the lowlands. Seeds of this tree planted in nursery beds at Los Baños germinated normally, but the seedlings grew poorly despite cultural treatments. Eventually they all died. Identical failures were frequently experienced in the following years, and seeds of *Pinus insularis* which were sent to South Africa and the Hawaiian Islands were no more successful when planted in the lowlands, although they thrived at higher elevations. This evidence appeared to indicate that *P. insularis* was not adapted to the climate of the torrid lowlands. However, potted seedlings from the nursery at Baguio, Mt. Province, Philippine Islands, located within the natural highland range of this species, were planted at Los Baños (in the lowlands) in 1919, 1922, 1927 and again in 1929. These trees thrived from the very beginning. They have averaged over a meter a year in height growth ever since, a growth-rate which is nearly twice that of the pine in its natural range. Subsequent use of soil collected from around the roots of these successful trees in the nursery at Los Baños has wholly eliminated the former difficulties.

Although it does not concern a nursery failure, no account of these remarkable evidences of the dependence of trees on a biological soil factor would be complete without reference to the first case of such dependence ever recorded. In this classical example, Melin (12) demonstrated that the growth of pine and spruce in newly drained peat bogs in northern Sweden was dependent upon the introduction into those bogs by the wind of the spores of mycorrhizal fungi. Growth of seedlings in the bogs was found to be directly proportional to the number and development of mycorrhizae. Lacking infection by mycorrhizal fungi, the seedlings invariably died. Thus the evidence pointed to these fungi as the specific biological factor which was necessary for the survival of trees in drained peat bogs.

Brief mention may also be made of several other reports of seedling difficulties in which the evidence points to a biological soil factor (probably mycorrhizae). In South Australia Samuels (17) attributed the failure or unsatisfactory results of the seed-spot method of reforestation to the absence or sporadic occurrence of mycorrhizal fungi in the seeded areas. In England, Stevens (18) reported that the broadcast seeding method is eminently successful if practiced shortly after logging; but if a period of years is allowed to pass before seeding, the method is unsuccessful. In this connection it is known that mycorrhizal fungi soon disappear from the soils of cleared woodlands (16). According to Laing (11), it is common knowledge among foresters that seedlings in newly established nurseries in England do poorly during their first year, when they lack mycorrhizae. As the mycorrhizal fungi become established (probably by wind-blown spores from surrounding woodlands), the seedlings first commence normal growth.

SIGNIFICANCE OF THESE FAILURES

The first four of these examples in particular afford striking support for the view that mycorrhizae are essential to the life of trees. Unfortunately the evidence is circumstantial only. Inoculation of seedbeds with *pure cultures* of mycorrhizal fungi would alone have supplied incontrovertible proof (such as was adduced long ago for the nodule-bacteria of

legumes) that mycorrhizal fungi rather than other soil organisms were responsible for these remarkable recoveries. Nevertheless, the failures point unquestionably to the need for recognizing the lack of appropriate soil organisms as a limiting factor to the growth of trees in prairie regions.

Experimental

An opportunity to obtain freshly collected soil from the American prairies prompted the writer to initiate a small and exploratory study of the mycorrhizal factor in afforestation. It was planned to learn: (1) whether ectotrophic[2] mycorrhizal fungi are lacking in the soils of that region, and (2) whether these fungi in reality are the specific soil organisms that are essential for tree growth in such soils.

The soil was collected by Dr. Paul A. Vestal and Mr. Richard H. Goodwin of Harvard University from a treeless tract 19 miles east of Cheyenne, Wyoming, and immediately shipped to the writer by express. It was evenly mixed with coarse silica sand (two-thirds sand) and potted in glazed bottomless one-gallon jugs. Twenty germinated seeds of *Pinus strobus* L. were planted in each of six containers in August, 1934. The containers were placed in a water bath and the whole protected from contamination by spores of mycorrhizal fungi by enclosing the water tank in glass and filtering through cotton the air forced into the chamber so formed. The plants were watered frequently with a slight excess of distilled water, and the excess allowed to drain free of the system. Daylight was supplemented by 4½ hours of radiation from three 200-watt tungsten filament lamps suspended 10 inches above the seedlings.

By early November the seedlings in all six containers were small, yellow, and unthrifty in appearance. They had already set bud and gone into winter dormancy, a process which occurs much earlier in pine seedlings grown under conditions of low nutrient availabilities than in those with larger supplies of nutrients. Examination revealed that mycorrhizae were wholly absent.

The seedlings in three of the containers were then inoculated with pure cultures of the following mycorrhizal fungi: *Boletus luteus, Boletinus pictus, Lactarius deliciosus* L., *L. indigo* (cultured by Dr. K. D. Doak), and *Mycelium radicis nigrostrigosum* (6, 7). The plants were kept at low temperatures during the winter (fluctuating with the temperatures in the unheated greenhouse, the water being removed from the water-bath). In March the containers were removed from the glass enclosure and placed in the open air of a heated greenhouse.

Between the first of April and the last of May differences in the appearance and size of the seedlings became apparent. The new needles on the inoculated seedlings in pot 2 were the first to become dark green and to elongate rapidly. These changes appeared later in the inoculated seedlings in pot 3, and finally in pot 4. The new needles on the seedlings in the uninoculated pots, except in pot 5, remained yellow and short throughout the experiment. Early in May the yellow color reappeared in the leaves of seedlings in pot 2 as well as in those in pot 5.

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[2]*Ectotrophic* mycorrhizae (5) are "absorbing" organs in which the fungus forms a parenchyma-like mantle over the exterior of the root, effectively separating it from the soil. The fungus likewise normally penetrates between and separates from one another the cells of the primary cortex. In the other type of fungus roots, known as *endotrophic* mycorrhizae, the fungus does not form an exterior mantle but grows chiefly within rather than between certain of the cells of the primary root cortex.

The plants were harvested between May 27 and June 5, at which time the root systems were examined for mycorrhizae and silhouette photographs (Figs. 1-3) were taken. In pot 2 the short roots, many of which had been mycorrhizal, were all dead. In pot 3 approximately 30 per cent of all short roots were mycorrhizal. They were produced by *Boletus luteus* except for a few dozen which were formed by *Lactarius deliciosus* at the point at which the inoculum was introduced into the soil. In pot 4, in which *Boletinus pictus* had been introduced, the mycelium spread only slowly from the point of introduction. Thirteen of the twenty seedlings in the pot had upwards to 90 per cent of their short root converted to mycorrhizae (Fig. 2).

The plants were dried at 65 degrees C. and the individual weights of the roots and tops of each plant determined. From each pot four seedlings whose weights were close to the mean weight of all seedlings in each pot were analyzed (tops and roots separately) for nitrogen, phosphorus, and potassium. The methods of analysis used were developed for resinous materials by Professor P. R. Gast (3) at the Harvard Forest. For nitrogen a modified form of the micro-Kjeldahl method of Pregl (13) was used. After perchloric acid digestion, phosphorus and potassium were determined colorimetrically (2,9). The results are presented in Table 1.

TABLE 1

DRY WEIGHTS, ROOT-SHOOT RATIOS, AND NITROGEN, PHOSPHORUS, AND POTASSIUM CONTENTS OF MYCORRHIZAL AND NON-MYCORRHIZAL WHITE PINE SEEDLINGS RAISED IN PRAIRIE SOIL, INCLUDING COMPARISONS WITH LOWEST PERCENTAGES OF N, P, AND K HITHERTO RECORDED FOR THIS PINE IN SOILS AND IN NUTRIENT SAND-CULTURES

		Root-	Nitrogen		Phosphorus		Potassium	
Pot No.	Av. dry weights of seedl. in mg.	shoot ratio	mg per seedl.	% of dry wt.	mg per seedl.	% of dry wt.	mg per seedl.	% of dry wt.
1	360.7 ± 8.7[1]	1.024	2.51	.695	.268	.0742	1.94	.539
2[2] (inoc.)	428.5 ± 19.2	.9176	3.01	.703	.566	.1320	1.93	.450
3 (inoc.)	448.4 ± 9.9	.672	5.39	1.202	.849	.1893	3.47	.775
4 (inoc.)	360.9 ± 4.8	.892	4.62	1.280	.729	.2021	2.57	.713
5	300.0 ± 7.0	1.365	3.16	1.056	.229	.0762	1.04	.347
6	301.4 ± 4.8	1.024	2.40	.795	.211	.0700	1.17	.390
Averages mycorrhizal seedl., pots 3, 4	**404.6**	**.782**	**5.00**	**1.241**	**.789**	**.1957**	**3.02**	**.744**
Averages non-mycorrhizal seedl., pots 1, 5, 6	**320.7**	**1.138**	**2.69**	**.849**	**.236**	**.0735**	**1.38**	**.425**
Lowest values hitherto recorded for 3-months white pine seedlings grown in any soil.[3]				1.081		.0825		.335
Per cent of N and P in 3-months white pine seedlings grown in nutrient sand-cultures in which these elements were individually omitted; all other elements being optimal.[4]				.720		.1040		**None available**

[1]Standard error of mean.

[2]In pot 2 mycorrhizae were produced early in the season but at the time of pulling all were dead as evidenced by complete suberization of cortex. The values for seedlings in this pot are intermediate and they are excluded from the averages.

[3]N and P values from seedlings grown in Ridge soil, Black Rock Forest, Mitchell (13, Table 7; P values not reported); K values from seedlings grown in very infertile soil (Mitchell, unpublished).

[4]Described (Mitchell, 13, Tables 3, 4 and 14) but, except for N values, unpublished results of Mitchell.

The facts that are of outstanding interest in this table are: (1) the marked increase in the absorption of nitrogen, potassium, and especially phosphorus by mycorrhizal plants (86 per cent more N, 75 per cent more K, and 234 per cent more P than in non-mycorrhizal plants), and (2) the obvious starvation of non-mycorrhizal plants as revealed by comparison with the lowest known percentage contents of these elements in white pine seedlings grown (a) in other infertile soils, and (b) in nutrient sand-cultures in which nitrogen or phosphorus was completely omitted.

It is believed that this evidence is conclusive in showing that the white pine seedlings grown in this prairie soil did not obtain sufficient nutrients to support normal growth when mycorrhizal fungi were absent.

Significance and Application in Afforestation

These results, although few and exploratory in scope, when viewed in the light of the nursery failures described above, would seem to establish (1) that mycorrhizal fungi are lacking in the soil of the American prairies (see Harvey, 4) in common with those of other unforested regions (except where trees have been introduced as transplants), (2) that in the absence of mycorrhizal fungi the absorption of nutrient elements by trees is apt

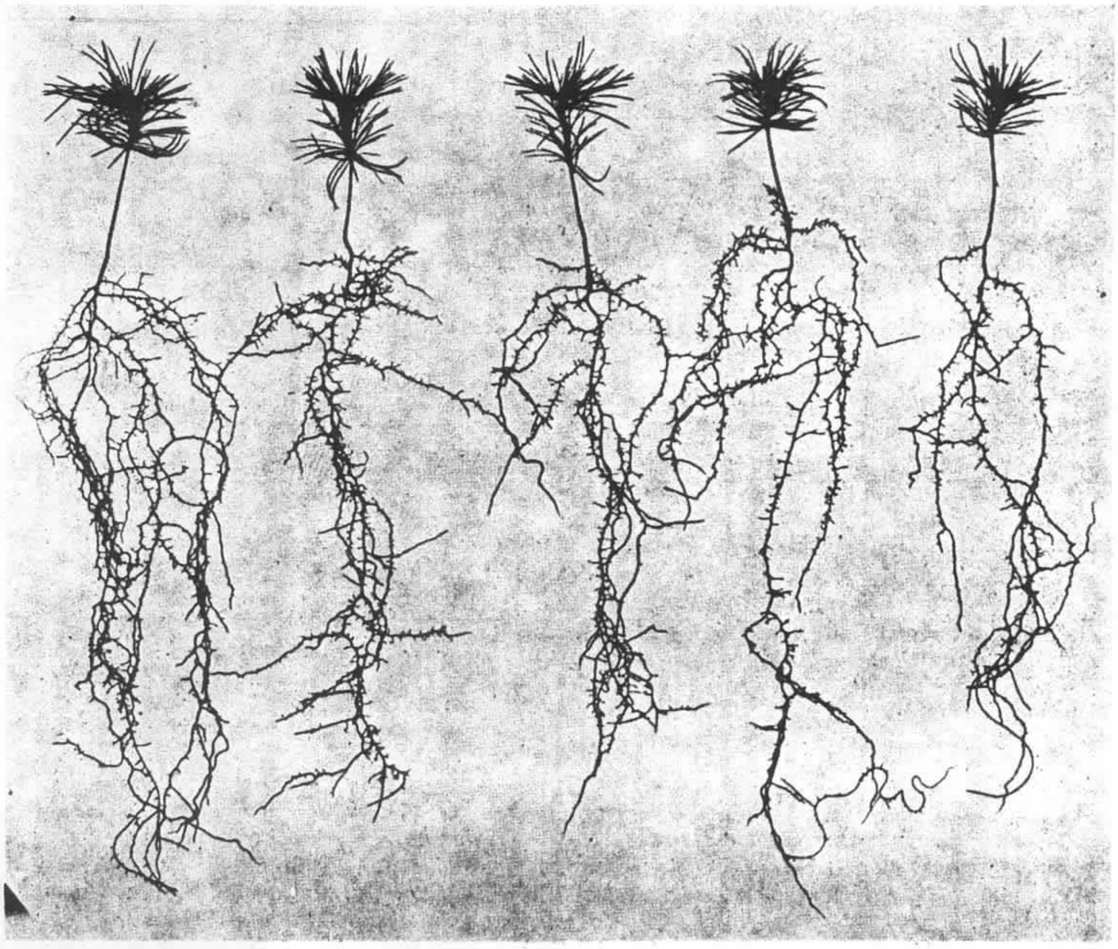

Fig. 1.—Non-mycorrhizal seedlings of *Pinus strobus* L. grown in prairie soil (pot 1).

to be inadequate to support normal growth, and (3) that mycorrhizal fungi constitute the specific biological factor which is necessary for the survival of trees in prairie regions.[3]

This knowledge may be put to immediate practical use in afforestation practice in prairie regions. When trees are transplanted from one region to another, destructive pathogens as well as mycorrhizal fungi are also transplanted to the new region. Both types of organisms are also transported to new regions when soil is used as the medium for introducing mycorrhizal fungi into new nurseries (see descriptions of Australian, Rhodesian, and Philippine nursery failures). To exclude the pathogens new nurseries and afforestation projects should be started using seeds only (already recommended for American projects). But if the trees in these nurseries are to survive, mycorrhizal fungi must be introduced artificially. Pure cultures of these fungi[4] or,

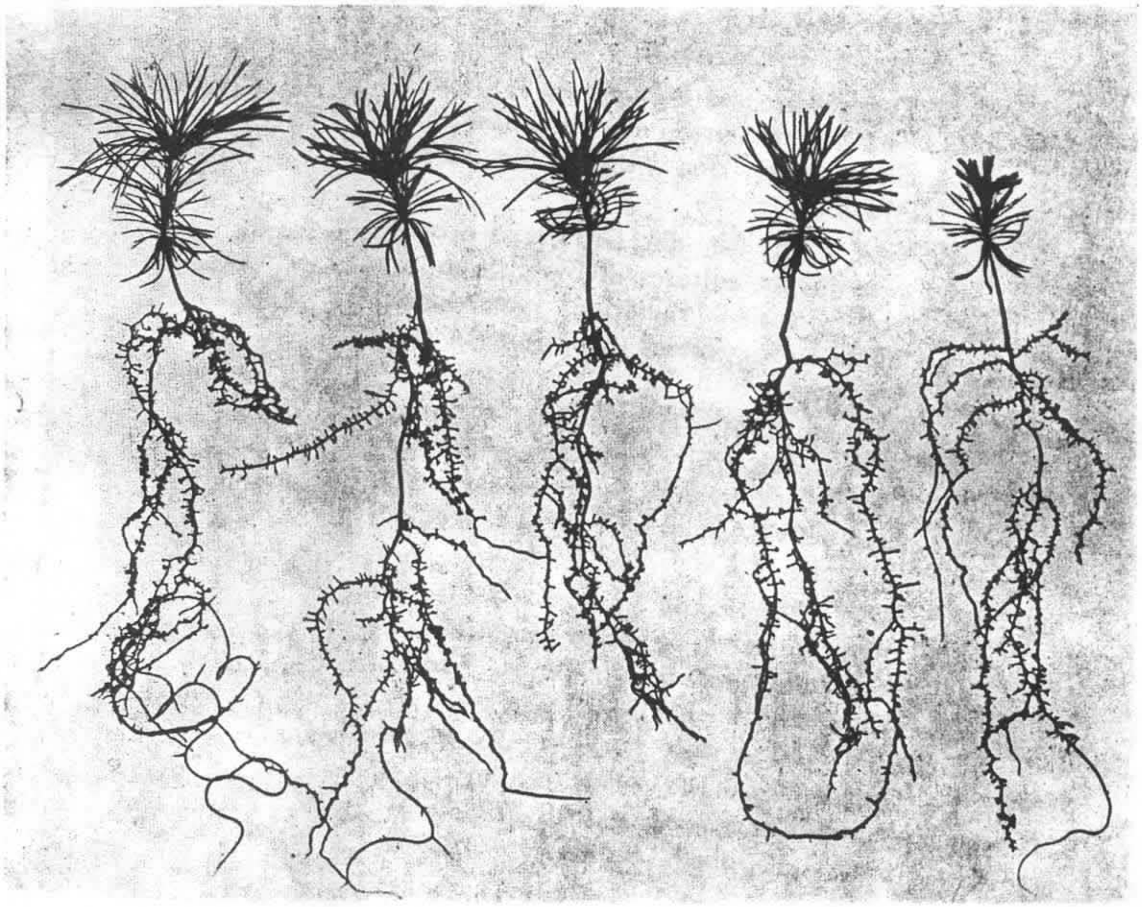

Fig. 2.—Four mycorrhizal and one (right) slightly mycorrhizal seedlings of *Pinus strobus* L. grown in inoculated prairie soil (pot 4).

[3]These results, in common with others (8), modify fundamentally the theory of mycotrophy in trees as it is now generally accepted. They will be discussed in this connection in a subsequent publication.

[4]The identities of the fungi producing endotrophic mycorrhizae are unknown, and with one exception among trees, they have not been obtained in culture.

if techniques can be developed, their spores of fructification, can alone be used for this purpose.

The application of these principles to nursery practice in afforestation projects will make possible (1) the exclusion of pathogens, (2) the survival of trees planted in areas where soil and moisture conditions are suitable, (3) the accumulation of exact data on the relative growths of trees inoculated with different species of mycorrhizal fungi and planted in different habitats.

Since it is known that different species of mycorrhizal fungi vary in their ability (1) to produce mycorrhizae with different trees, (2) to survive in different habitats, and (3) to stimulate tree growth, the early accumulation of field data on these variables is of vital importance to the success of afforestation projects.

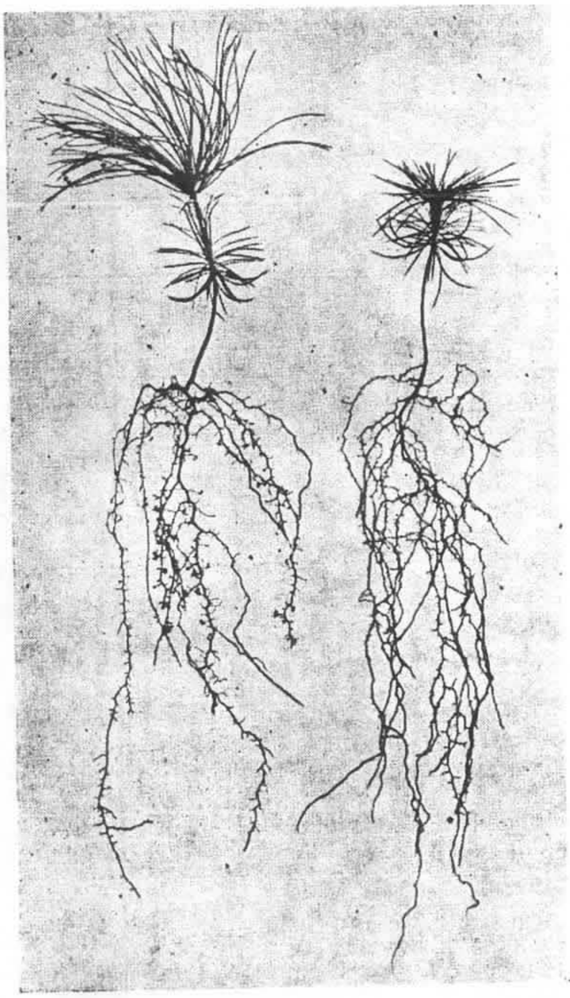

Fig. 3.—Mycorrhizal (left; pot 3) and non-mycorrhizal (right; pot 6) seedlings of *Pinus strobus* L. grown respectively in inoculated and uninoculated prairie soil.

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